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STREPTOCOCCAL AND STAPHYLOCOCCAL L FORMS IN
VIVO

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and on the concept that readily controlled studies performed by inoculating specific organisms avoids confusion arising from difficulties in identifying the parentage of L forms recovered from natural infections of varying bacterial etiology.

These studies answered five questions regarding the role of L forms in these induced infections. First, these variants that appeared in vivo probably represented spontaneously occurring variants, and were not produced by host mechanisms such as lysosomal enzymes. Second, they appeared to survive in vivo in an osmotically unfavorable milieu because of some stabilizing substance in exudate, probably a polyamine. Third, penicillin did not induce L form production in vivo. Fourth, no evidence that L forms behave as bacterial persisters was found. Fifth, L forms themselves were non-infective.

Thus, these studies suggest little or no role for L forms in the pathogenesis of bacterial disease.

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STREPTOCOCCAL AND STAPHYLOCOCCAL L FORMS IN VIVO

FINAL REPORT

by

Edward A. Mortimer, Jr., M.D.

May 1975

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SUMMARY

L forms are viable, reproducing bacterial variants, lacking cell wall components except the protoplast membrane. Interest in these variants stems from the possibility that they represent bacterial forms that persist, even in the presence of anti-cell wall antibiotics.

The basis of these studies is that L forms can be recovered from streptococcal and staphylococcal infections in animals, and on the concept that readily controlled studies performed by inoculating specific organisms avoids confusion arising from difficulties in identifying the parentage of L forms recovered from natural infections of varying bacterial etiology.

These studies answered five questions regarding the role of L forms in these induced infections. First, these variants that appeared in vivo probably represented spontaneously occurring variants, and were not produced by host mechanisms such as lysosomal enzymes. Second, they appeared to survive in vivo in an osmotically unfavorable milieu because of some stabilizing substance in exudate, probably a polyamine. Third, penicillin did not induce L form production in vivo. Fourth, no evidence that L forms behave as bacterial persisters was found. Fifth, L forms themselves were non-infective.

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This final report covers the entire period in which this grant was in progress.

L forms are viable, reproducing bacterial variants that lack all components of the cell wall, and having only the protoplast membrane as a surrounding envelope. As such, these organisms, although mechanically and osmotically quite fragile, are insusceptible to commonly used antibiotics, such as penicillin, the action of which is upon the cell wall. Further, they appear to retain all of the biologic activities of the parent organism, other than the ability to synthesize a cell wall.

L forms have been identified for many years as variants of a variety of bacteria, both gram negative and gram positive. They may appear spontaneously on appropriate media, and in the laboratory may be induced from the parent bacterium by various agents, such as penicillin, that act upon the cell wall.

The possibility that they might be involved in the pathogenesis of human infection has been conjectured by many. Specifically, it has been considered that they might function as persisting forms of bacteria which, under the proper circumstances, might revert to the parent form inducing a recrudescence of infection. Further, their insusceptibility to anti-cell wall antibiotics might explain the persistence of infection in the presence of these therapeutic agents.

Studies of the role of bacterial L forms in the pathogenesis of disease are difficult for several reasons. First, the organisms are fragile and difficult to cultivate in vivo. Second, maximum confusion has existed because differentiation of L forms from other microscopic structures, such as platelets or other granule-like organelles in tissue, is extremely difficult, if not impossible. Third, L forms must be cultured in medium containing horse serum or other similar ingredients that cannot be sterilized by heat; accordingly media contamination with a variety of organisms, even including contaminating L forms, is an ever present danger.

The present studies were based on the observation, made by the principal investigator, that L forms could be recovered readily from mice infected with group A streptococci (Proc. Soc. Exp. Biol. Med. 119:159, 1965). These L forms were clearly shown to have been derived from the parent streptococcus immunobiologically and by reversion. They were also shown without question to have existed in vivo in the infected

animals and not an artifact of the culture techniques. This experimental model therefore seemed to get around the problem of confusing L forms with other tissue structures, inasmuch as colonies readily grew to macroscopic size on appropriate medium. Further, the problem of contamination and confusion with other organisms was eliminated by virtue of the fact that these organisms could readily be identified as being derived from the parent streptococcus. Therefore it seemed that this model should permit direct study of their possible role in the pathogenesis of bacterial infection. These studies are reviewed in greater detail in the publication listed first in the bibliography. In brief, five questions were asked.

The first question was that of how these variants were produced in vivo. It seemed logical that they occurred as a consequence of interference with the synthesis of the cell wall by lysosomal enzymes. This was studied in two ways. First, strains of group A streptococci known to convert readily to the L form in vivo were treated with preparations of lysosomal enzymes, produced by disruption of cells and intracellular particles. Such treatment failed to produce L forms at a greater rate than spontaneous variation in control preparations. Second, it was hypothesized that, if indeed lysosomal enzymes were responsible for the production of L forms in vivo, modification of the reticuloendothelial system in infected mice might alter rates of production of L forms. Pharmacologic agents known to enhance on the one hand and to inhibit on the other hand reticuloendothelial activity were administered to infected mice with no alteration in the yield of L forms compared to controls. It was therefore concluded that the answer to the first question was that these L forms appeared as spontaneous variants in infected mice, and were not a consequence of the defense mechanisms of the mice.

The second question asked was the mechanism by which L forms survived in vivo, inasmuch as in vitro they require an osmotic milieu three or four times that existing in man and animal. The most likely explanation was thought to be the presence of some stabilizing substance in inflammatory exudate. Since it was known that serial dilution of L forms in 0.9% saline resulted in their lysis, experiments were undertaken in which L forms were serially diluted in sterile peritoneal exudate from mice. Serial dilution in saline was used as controls. The results of these experiments clearly indicated that there is some stabilizing substance in exudate. The nature of this substance was not determined, but it was probably a polyamine.

The third question asked was whether penicillin would induce L forms in vivo in infected animals. To accomplish this, mice were infected intraperitoneally with group A streptococci and treated with penicillin. The sum and substance of many experiments is that there was not a shred of evidence that penicillin produced a higher yield of L forms from infected mice compared to controls. Similar experiments were conducted with staphylococci which readily produce arthritis in hamsters. Streptococcal impetigo in hamsters was also studied in similar fashion, and the results of all of the experiments were the same. Thus, it was concluded that under the conditions of these experiments penicillin does not induce the production of streptococcal and staphylococcal L forms in vivo.

The fourth question asked was whether these bacterial variants might persist in infections from which the animals had recovered to the point that the full bacterial forms could no longer be recovered. In brief, employing the same models, no evidence of the presence of L forms subsequent to disappearance of the parent bacterium from an infected lesion was found.

The fifth question asked was whether L forms were infective. Streptococcal L forms are difficult to grow in liquid medium, but with repeated transfer in media containing less and less agar growth can ultimately be achieved. Therefore, preparations of L forms in liquid media were injected into animals, some of whose defenses had been compromised by pharmacologic agents known to interfere with the reticuloendothelial system. Serial sampling of blood and peritoneal fluid showed that these organisms disappeared within a very few hours. Thus, no evidence was obtained that these organisms are infective.

The final conclusion from these experiments is that L forms may be vastly over-rated as possible contributors to the pathogenesis of bacterial infections in man. It is the belief of the principal investigator that they do exist in vivo, but that they represent spontaneous variants of no pathogenetic consequence. They were never present in the absence of the parent bacterium in far greater numbers, and in the course of recovery from infection in animals the L forms disappeared in parallel with the parent bacterium. Thus, at least with streptococcal and staphylococcal infections, these experiments would seem to be significant in that the physician need not be concerned about these penicillin-resistant variants contributing to disease.

In the course of these studies several related experiments were conducted. A hamster model for impetigo was devised (Ref. 2). It was also shown that the capsular polysaccharide (hyaluronic acid) was produced by L forms of group A streptococci, if the

parent bacterium produced this substance (Ref. 3). It was also shown that the previous suggestion that the ability of group A streptococci to produce bacteriocines was associated with the ability to produce acute glomerulonephritis in man was invalid (Ref. 4).

Other studies, unpublished as yet, have shown that members of a family in which one child has impetigo are likely to be colonized at many different sites, not only in the respiratory tract but also on the skin, with the same group A streptococcus. Studies of streptococcal impetigo in hamsters in which super infection with a penicillinase-producing streptococcus was induced have provided some suggestion that the penicillinase may interfere with the action of penicillin on the group A streptococcus. In the course of these studies an outbreak of streptococcal glomerulonephritis in northern New Mexico was also studied, as well as an outbreak of group A streptococcal infections in newborn nurseries.

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